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Control Analysis for Autonomously Oscillating Biochemical Networks

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ABSTRACT It has hitherto not been possible to analyze the control of oscillatory dynamic cellular processes in other than qualitative ways. The control coefficients, used in metabolic control analyses of steady states, cannot be applied directly to dynamic systems. We here illustrate a way out of this limitation that uses Fourier transforms to convert the time domain into the stationary frequency domain, and then analyses the control of limit cycle oscillations. In addition to the already known summation theorems for frequency and amplitude, we reveal summation theorems that apply to the control of average value, waveform, and phase differences of the oscillations. The approach is made fully operational in an analysis of yeast glycolytic oscillations. It follows an experimental approach, sampling from the model output and using discrete Fourier transforms of this data set. It quantifies the control of various aspects of the oscillations by the external glucose concentration and by various internal molecular processes. We show that the control of various oscillatory properties is distributed over the system enzymes in ways that differ among those properties. The models that are described in this paper can be accessed on <http://jjj.biochem.sun.ac.za>.

INTRODUCTION

Periodic phenomena are widespread in biology (for review see Hess, 2000), e.g., calcium waves (Rottingen and Iversen, 2000; Bootman et al., 2001), oscillations in neuronal signals (Rabinovich and Abarbanel, 1998), oscillations in cyclic AMP in the slime mould *Dictyostelium discoideum* (Halloy et al., 1998; Nanjundiah, 1998), yeast glycolytic oscillations (Ghosh and Chance, 1964), the circadian rhythm (Turek, 1998), and the cell cycle (Johnson et al., 1996; Mori and Johnson, 2000; Smaaland, 1996; Tyson and Novak, 2001). Periodic signals can be a function of time (glycolytic oscillations), space (striping in *Drosophila melanogaster* embryos), or both (*D. discoideum*, calcium waves, neuronal oscillations) depending on the mechanism of the oscillator. Some of these periodic phenomena are crucial for the living system in which they occur. Consequently, it should be important to identify the processes that determine these oscillations, if only to understand which molecular defects result in pathological disturbances in oscillations. The complexity of what determines biological oscillations has often been underestimated by attributing all control to a single pace-maker. In recent years it has become clear that the frequency and amplitude of biological oscillations can be controlled by more than one enzyme (Teusink et al., 1996a; Reijenga et al., 2001). However, oscillatory properties carry more information than frequency and amplitude, such as average value, waveform, and phase shift. All of these properties may be controlled by the molecular

biological processes in the system, and perhaps differentially so. The functional importance of oscillatory phenomena may reside in any of these properties or in their combinations. Calcium and endocrine oscillations appear to function as information-transfer pathways where the information is frequency rather than amplitude encoded (Goldbeter et al., 1990; Goldbeter, 1996; Bootman et al., 1996). The importance and the inherent complexity of the control of biochemical oscillations suggest that a systematic way of analyzing this control might be useful.

Metabolic Control Analysis (MCA) is a systematic method for analyzing the control of steady states. It quantifies the extent to which any parameter, but more notably all molecular processes, controls any steady-state variable within a metabolic pathway (Kacser and Burns, 1973; Heinrich and Schuster, 1996). Oscillations are dynamic and therefore do not exist in a steady state. Standard MCA cannot be applied to transient oscillations (but see Heinrich and Reder, 1991). An operational definition of a time-dependent control coefficient was introduced by Acerenza et al. (1989) as the relative change in a system variable at time t after a perturbation of a parameter at time zero, divided by the relative change in that parameter. It appears, however, that this time-dependent control coefficient is not useful for the characterization of autonomously oscillating systems, because its magnitude diverges as time progresses (Kholodenko et al., 1997; Demin et al., 1999). Neither standard MCA nor its extension proposed by Acerenza et al. and Heinrich and Reder (1991) can be applied to the steadily varying concentrations in a limit cycle oscillation (Kholodenko et al., 1997; Demin et al., 1999). In contrast, the frequency, amplitude, average value, waveform, and phase shift are time independent in such oscillations, and this should make it possible to develop an MCA-like approach for those properties. Parts of such an approach have

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been developed (Westerhoff et al., 1990; Bier et al., 1996; Kholodenko et al., 1997; Demin et al., 1999; Reijenga et al., 2001), but all but one of these focused on frequency. The principles of a more general approach have been put forward theoretically (Kholodenko et al., 1997), but elaborations of how it should be implemented in actual systems have been lacking. Here we wish to make the approach operational by applying it to an actual system, i.e., that of yeast glycolytic oscillations.

Yeast glycolytic oscillations have been studied both in cell-free extracts and in whole cells for almost 40 years (e.g., Ghosh and Chance, 1964; Richard et al., 1996; Teusink et al., 1996b). Yeast cells show transient oscillatory behavior after starvation of the cells and subsequent addition of glucose (Chance et al., 1964). The make-up of the cells determines the stability of the steady state and the distinction between transient oscillations and sustained (limit cycle) oscillations. When cells are harvested at the diauxic shift (shift from using glucose to using ethanol as a carbon source), the cells are prone to sustained oscillations, monitored macroscopically by measuring NADH fluorescence in populations of cells (Richard et al., 1994, 1996; Dano et al., 1999). To monitor these sustained oscillations, synchronization of the cells is crucial. The cells are synchronized by acetaldehyde, provided the latter is partly trapped, e.g., by added cyanide. Mathematical models of yeast cells with synchronizing glycolytic oscillations (Bier et al., 2000; Wolf and Heinrich, 2000) are realistic enough to serve as a silicon (Westerhoff, 2001) replicon of a real and experimentally accessible oscillatory system. This presents us with the possibility to use the models to develop, illustrate, and, to some extent, test new metabolic control analysis for realistic biological oscillations.

The aim then is to develop a systematic approach similar to MCA for the analysis of what controls autonomous oscillations. The notion elaborated in this paper is that the control of autonomous oscillations can be analyzed by considering the Fourier spectrum of the individual fluxes and concentrations. Laws that relate various control properties are derived and illustrated. Focus is on the five most obvious characteristics of an oscillating property, i.e., frequency (ω), average value (A_0), amplitude (A_n), waveform (A_2/A_1), and phase (φ).

MATERIALS AND METHODS

Theory and definitions

Metabolic control analysis

MCA quantifies the extent to which an enzyme i controls a steady-state variable X . Control coefficients are defined as the relative change in that steady-state variable upon a relative change in the activity (v_i) of the enzyme i ,

$$C_i^{X_j} = \frac{\partial X_j / X_j}{\partial v_i / v_i} = \frac{\partial \ln X_j}{\partial \ln v_i}.$$

The elasticity of an enzyme quantifies the extent to which the activity of that enzyme is changed by a parameter p , and reads, in mathematical terms,

$$\varepsilon_p^i = \frac{\partial \ln v_i}{\partial \ln p}.$$

Furthermore, the way a variable responds to a change in a parameter is given by the response coefficient,

$$R_p^{X_j} = \frac{\partial X_j / X_j}{\partial p / p} = \frac{\partial \ln X_j}{\partial \ln p}.$$

Combination of these three properties gives the combined response theorem,

$$R_p^{X_j} = \frac{\partial \ln X_j}{\partial \ln v_i} \cdot \frac{\partial \ln v_i}{\partial \ln p} = C_i^{X_j} \cdot \varepsilon_p^i.$$

Here, it is assumed that the parameter p affects the enzyme i only.

Fourier transformation

Any periodic signal of period T can be expanded into a trigonometric series of sine and cosine functions:

$$\begin{aligned} f(t) &= A_0 + \sum_{n=1}^{\infty} (a_n \cos n\omega t + b_n \sin n\omega t) \\ &= A_0 + \sum_{n=1}^{\infty} \sqrt{a_n^2 + b_n^2} \cdot (\cos \varphi_n \cdot \cos n\omega t \\ &\quad - \sin \varphi_n \cdot \sin n\omega t) \\ &= A_0 + \sum_{n=1}^{\infty} A_n \cdot \cos(n\omega t - \varphi_n), \end{aligned}$$

where A_0 is the average value of the signal, a_n and b_n are the amplitudes of the cosine and sine, respectively, of the n th component, ω is the eigenfrequency of the signal, A_n is the absolute amplitude of the n th component of the signal ($A_n = \sqrt{a_n^2 + b_n^2}$) and φ_n is the phase of the n th component of the signal ($\varphi_n = \arctan(b_n/a_n)$). For a virtually sinusoidal oscillation, the Fourier spectrum peaks at $n = 1$, and the other components can be disregarded. In general, the component amplitude in a potentially infinite Fourier series rapidly decreases with the number n . In this study, the amplitude will be studied in terms of the absolute amplitudes of the first and second component of the Fourier spectrum (A_1, A_2). The waveform is studied in terms of the ratio of the first and second components of the Fourier spectrum (A_2/A_1).

Methods

We used a numerical approach to calculate control coefficients, because limit cycle oscillations can only be analyzed analytically infinitely close to the Hopf bifurcation. All models were programmed in the metabolic modeling program GEPASI (Mendes, 1997). Fourier transformations were carried out and analyzed using MATHEMATICA (Wolfram, 1999). A data set was generated in GEPASI and imported in MATHEMATICA. Using Fourier transformations, an estimation of the average value of the data was made and this value was used to calculate the exact beginning and end of N number of periods (with N a natural number, usually ~ 10). Subsequently, a discrete Fourier transform was carried out on such a subset of the

data and the frequencies and amplitudes calculated. Furthermore, a reverse Fourier transform was made and compared to the original signal. The three main components were analyzed in terms of MCA.

To calculate the response of a variable toward a change in a parameter, that parameter was changed by both $+\delta p$ and $-\delta p$ around the reference state. For calculation of the control coefficients, the activities of the enzymes (V_{\max} or corresponding rate constants) were also changed by both $+\delta p$ and $-\delta p$ around the reference state. New limit cycles were computed, and the Fourier spectrum was taken. The response coefficient equals the slope of the curve when plotting $\ln X_j$ versus $\ln p$, whereas the control coefficient equals the slope of the curve when plotting $\ln X_j$ versus $\ln v_i$. Here, X_j can be any element of the Fourier spectrum (ω , A_0 , A_1 , A_2 , A_2/A_1 , and φ). The magnitude of the parameter change δp was balanced between linearity of the \ln - \ln plot (which usually requires small changes) and accuracy of determination of the change of the components in the Fourier spectrum.

Models

In this study, we used three models (Fig. 1, A–C), which are described below in terms of differential equations. The models can also be accessed and run on <http://ijj.biochem.sun.ac.za>. Further details on the models can be found in the original literature. For comparison, we have chosen to use the same symbols as used in the original literature.

Model 1: the PFK model by Goldbeter and Lefever (1972)

The phosphofructokinase (PFK) model is a core model that describes glycolysis solely in terms of the kinetics of the enzyme PFK (Fig. 1 A). The model consists of two variables α and γ , and three reaction rates,

$$\frac{d\alpha}{dt} = \sigma_1 - \sigma_M \Phi, \quad \frac{d\gamma}{dt} = \sigma_M \Phi - k_s \gamma,$$

where

$$\Phi = \frac{\alpha e(1 + \alpha e)(1 + \gamma)^2 + L\Theta \alpha e'(1 + \alpha e')}{L(1 + \alpha e')^2 + (1 + \gamma)^2(1 + \alpha e)^2}$$

includes the most important kinetic details of PFK. The variables α and γ denote the concentrations of substrate (ATP or fructose 6-phosphate) and product (ADP or fructose 1,6-bisphosphate), respectively. σ_1 denotes the constant injection rate of substrate, σ_M is the rate constant (or concentration) of PFK, and k_s is the rate constant for the sink of the product. Explanation of the other parameters can be found in the original literature. In the present study, a set of parameter values was used according to Goldbeter and Caplan (1976): $L = 10^6$; $c = 10^{-5}$; $e = e' = 0.9090909$; $\Theta = 1$; $\sigma_1 = 0.7$; $\sigma_M = 4$; $k_s = 0.1$.

Model 2: a core model by Bier et al. (2000)

Bier et al. describes glycolysis in terms of two variables, i.e., (internal) glucose and ATP (Fig. 1 B). The system is summarized by the following dynamical system:

$$\frac{dG}{dt} = V_{in} - k_1 GT,$$

$$\frac{dT}{dt} = 2k_1 GT - k_p \frac{T}{K_m + T},$$

where G and T denote the internal glucose concentration and the ATP concentration, respectively. V_{in} is the constant influx of glucose and k_1 is

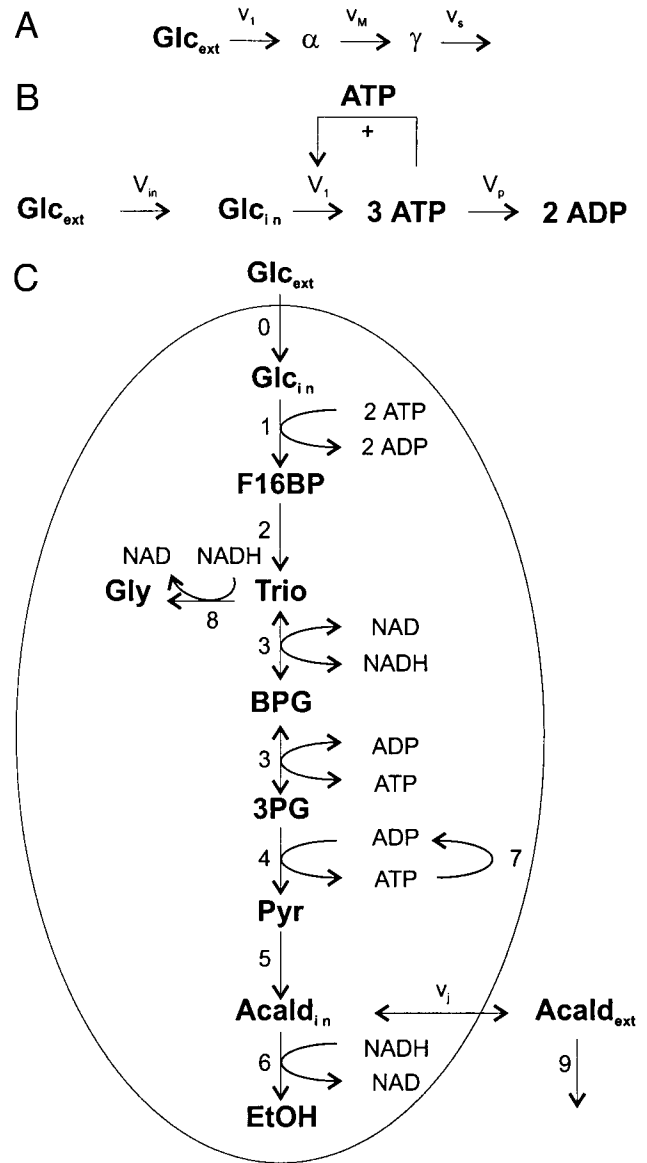


FIGURE 1 Reaction schemes for the models (A) 1, (B) 2 and (C) 3. See the text for explanation of the symbols and the rate equations.

the enzyme activity (or concentration) of PFK. There is a positive feedback, i.e., ATP stimulates its own production. Furthermore, ATP is broken down according to Michaelis–Menten kinetics. In this study, we used the following parameter set as a reference state: $V_{in} = 0.36$; $k_1 = 0.02$; $k_p = 6.0$; $K_M = 13.0$ (Bier et al., 2000).

Model 3: the nine-variable model by Wolf et al. (2000)

Wolf et al. have set up a minimum model of glycolysis in yeast that qualitatively describes the experimental observations of Richard et al. (1996) (Fig. 1 C). The model consists of lumped reactions and includes branches to glycerol and ethanol. Furthermore, it accounts for the diffusion of acetaldehyde across the plasma membrane, the trapping of acetaldehyde (the synchronizing agent) by cyanide, and the presence of more than one cell. Therefore, this model can describe intercellular synchronization. The

one-cell version of the model contains nine variables and the system is described by the following differential equations:

$$\begin{aligned}\frac{dS_1}{dt} &= J_0 - v_1, & \frac{dS_2}{dt} &= v_1 - v_2, \\ \frac{dS_3}{dt} &= 2v_2 - v_3 - v_8, & \frac{dS_4}{dt} &= v_3 - v_4, \\ \frac{dS_5}{dt} &= v_4 - v_5, & \frac{dS_6}{dt} &= v_5 - v_6 - J, & \frac{dS_6^{\text{ex}}}{dt} &= \varphi J - v_9, \\ \frac{dA_3}{dt} &= -2v_1 + v_3 + v_4 - v_7, & \frac{dN_2}{dt} &= v_3 - v_6 - v_8,\end{aligned}$$

with the rate equations,

$$\begin{aligned}J_0 &= \text{constant}, \\ v_1 &= \frac{k_1 S_1 A_3}{1 + (A_3/K_i)^n}, & v_2 &= k_2 S_2, \\ v_3 &= \frac{k_{\text{GAPDH}+} k_{\text{PGK}+} S_3 N_1 (A - A_3) - k_{\text{GAPDH}-} k_{\text{PGK}-} S_4 A_3 N_2}{k_{\text{GAPDH}-} N_2 + k_{\text{PGK}+} (A - A_3)}, \\ v_4 &= k_4 S_4 (A - A_3), \\ v_5 &= k_5 S_5, & v_6 &= k_6 S_6 N_2, \\ v_7 &= k_7 A_3, & v_8 &= k_8 S_3 N_2, \\ v_9 &= k_9 S_6^{\text{ex}}, & J &= \kappa (S_6 - S_6^{\text{ex}}),\end{aligned}$$

and two conserved moieties,

$$A = A_2 + A_3, \quad N = N_1 + N_2.$$

Variables and their meanings are: S_1 , glucose; S_2 , fructose-1,6-bisphosphate; S_3 , pool of the triosephosphates, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate; S_4 , 3-phosphoglycerate; S_5 , pyruvate; S_6 , intracellular acetaldehyde; S_6^{ex} , extracellular acetaldehyde; A_2 , ADP; A_3 , ATP; N_1 , NAD; N_2 , NADH. The parameter values that were used in this study are listed in Table 1 (Wolf et al., 2000).

RESULTS

Fourier spectra

Fourier analysis is not yet standard in metabolic control software. Therefore, we first developed a routine in MATHEMATICA that enabled us to take a Fourier spectrum of a set of data generated in the metabolic modeling program GEPASI. The bold line in Panel A of Fig. 2 shows an autonomous oscillation in the concentration of variable α calculated for model 1 of glycolysis. The filled diamonds in Panel B give the corresponding discrete Fourier spectrum. The zero frequency component of amplitude 70 mM corresponds to the time-average value of the concentration variable. The first Fourier component represents a sinusoidal oscillation with an amplitude of approximately half that average. That the oscillation has a form that deviates from

TABLE 1 Parameter values for model 3 by Wolf et al. (2000)

Parameters	Value
J_0	50.0 mM · min ⁻¹
k_1	550.0 mM ⁻¹ · min ⁻¹
K_i	1.0 mM
k_2	9.8 min ⁻¹
$k_{\text{GAPDH}+}$	323.8 mM ⁻¹ · min ⁻¹
$k_{\text{GAPDH}-}$	57823.1 mM ⁻¹ · min ⁻¹
$k_{\text{PGK}+}$	76411.1 mM ⁻¹ · min ⁻¹
$k_{\text{PGK}-}$	23.7 mM ⁻¹ · min ⁻¹
k_4	80.0 mM ⁻¹ · min ⁻¹
k_5	9.7 min ⁻¹
k_6	2000.0 mM ⁻¹ · min ⁻¹
k_7	28.0 min ⁻¹
k_8	85.7 mM ⁻¹ · min ⁻¹
κ	375.0 min ⁻¹
φ	0.1
A	4.0 mM
N	1.0 mM
n	4

a simple sinus (cf. Fig. 2 A), is consolidated by the presence of higher-order Fourier components, e.g., the one at a frequency near 0.62 min⁻¹ (cf. Fig. 2 B). Because the third and higher-order components were small, we shall further focus

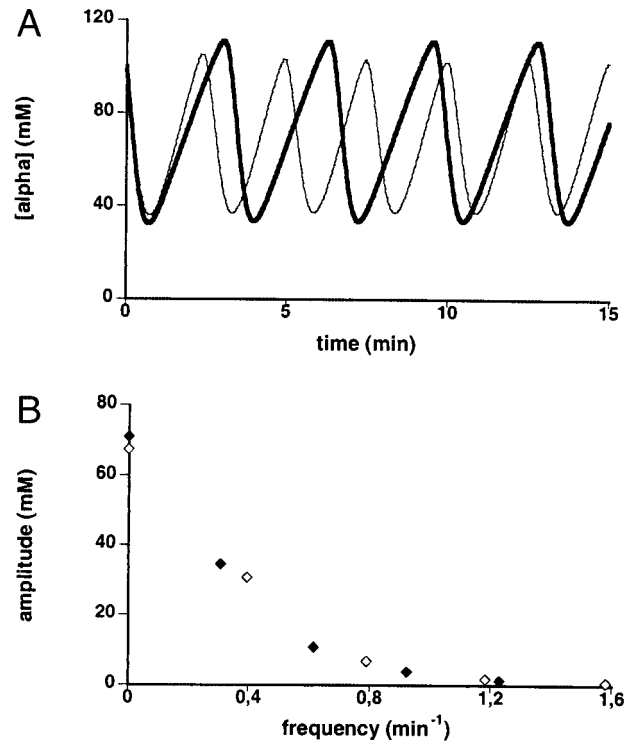


FIGURE 2 Output of the PFK model by Goldbeter and Lefever (1972). See the text for parameter values. (A) The concentration of metabolite α in time. (B) The corresponding Fourier spectrum. The bold line and the filled diamonds reflect the oscillation at $[\text{Glc}]_{\text{ext}} = 1$ mM, the thin line and the open diamonds reflect the oscillation at $[\text{Glc}]_{\text{ext}} = 1.5$ mM.

TABLE 2 Fourier transformations for model 1

Fourier elements	[Glc] _{ext} = 1.0			[Glc] _{ext} = 1.05		
	ω (min ⁻¹)	α (mM)	γ (mM)	ω (min ⁻¹)	α (mM)	γ (mM)
A_0		70.98	7.00		71.18	7.35
First component (A_1)	0.31	34.52	10.59	0.32	34.91	10.96
Second component (A_2)	0.62	10.77	5.84	0.63	10.56	5.83
Third component (A_3)	0.92	3.72	2.60	0.95	3.51	2.48
Fourth component (A_4)	1.23	1.24	1.00	1.26	1.13	0.91

Two different values for the external substrate concentration [Glc]_{ext} were used (1.0 and 1.05 mM). Parameter values were as described in the text.

on the ratio of the amplitudes of the second- and the first-order Fourier components as a measure of the waveform of the oscillations.

In the original model by Goldbeter and Lefever (1972) the substrate injection rate was constant (σ_1). To determine the response of the system to external glucose, the kinetics of this first reaction were adapted to reflect mass action of the external glucose ($\sigma_1 = k_1 * [\text{Glc}]_{\text{ext}}$). The external glucose concentration was then increased by 50% and the resulting oscillations calculated (Fig. 2 A, *thin line*). In accordance with experimental expectations (Hess and Boiteux, 1973; Reijenga et al., 2001), the frequency of the oscillations increased. The Fourier spectrum (in Fig. 2 B, *diamonds*) reflected the change in frequency and revealed that, also, the waveform had been changed slightly, which was less noticeable in the time domain representation of Fig. 2 A.

The effect of the external glucose concentration on the oscillation depended (nonlinearly) on the extent to which the glucose concentration was changed. To move away from this effect, we decided to focus on the effects of small modulations of the glucose concentration, much in the vein of MCA. Therefore, the glucose concentration was increased by a mere 5%. The elements of the two Fourier spectra are listed in Table 2. This table testifies to a change in both frequency and amplitude of the different Fourier components, and in the average value of the variables upon a change in external glucose.

Response of the oscillation to external glucose in terms of Fourier response coefficients

From the time domain representation of Fig. 2 A, it is not immediately obvious how to describe the response of the oscillation to an increase in external glucose concentration. If one were to determine the change in the concentration variable α and divide that by the change in external glucose concentration, as should be done for a traditional response coefficient, a time-dependent result would appear that, itself, oscillated with a sizable amplitude even for very small modulations of the glucose concentration (not shown; Kholodenko et al., 1997). The frequency domain presenta-

TABLE 3 Response coefficients of the external substrate on the Fourier spectrum for the dynamic behavior of the concentrations of metabolites of model 1

$R_{\text{gluc}}^{Y_X}$	ω	A_0	A_1	A_2	A_2/A_1
α	0.50	0.06	0.24	-0.39	-0.63
γ	0.50	1.00	0.71	-0.01	-0.72

tion of Fig. 2 B, however, suggests how to characterize the control external glucose exerted on the oscillations, i.e., in terms of the effect on frequency and on the amplitudes of the various Fourier components. For model 1, we therefore quantified the response of the Fourier spectrum to a change in external glucose in terms of such response coefficients. The Fourier response coefficient is defined as

$$R_p^X = \frac{\partial \ln X}{\partial \ln p},$$

where p is a parameter (i.e., external glucose) and X is a dependent variable (i.e., any of the elements of the Fourier spectrum; ω , A_0 , A_1 , A_2 , A_2/A_1 , and φ). Table 3 shows the response coefficients for the different elements of the Fourier spectrum, for the two metabolites of the system (α , γ). As quantified by this table, external glucose affected frequency, average value, amplitude, and waveform of the oscillations. Frequency and waveform of the oscillations of the two metabolites were affected to virtually the same extent (even though the waveforms of the two variables differed considerably), whereas the response differed for the average value A_0 and the amplitude of the first and second Fourier component. Furthermore, for the amplitude of the second Fourier component (A_2), the response coefficients were negative, whereas, for the other elements of the Fourier spectrum, the response coefficients were positive.

Dependence of the oscillation on kinetic parameters

We calculated the control of the enzymes on the oscillations for the three models. We changed the kinetic parameters (i.e., the activity) of the individual enzymes and determined the Fourier spectra. The control coefficients are listed in Tables 4, 5, and 6. The tables show that, for all three

TABLE 4 Control coefficients of the enzymatic steps on the Fourier spectrum for model 1

Enzymatic step (i)	α					γ				
	C_i^ω	$C_i^{A_0}$	$C_i^{A_1}$	$C_i^{A_2}$	$C_i^{A_2/A_1}$	$C_i^{A_0}$	$C_i^{A_1}$	$C_i^{A_2}$	$C_i^{A_2/A_1}$	
σ_1	0.51	0.06	0.24	-0.39	-0.63	1.00	0.71	0.01	-0.70	
σ_M	0.41	-0.66	-0.17	0.52	0.69	0.00	0.19	0.76	0.57	
k_s	0.08	0.60	-0.07	-0.11	-0.04	-1.00	-0.90	-0.75	0.15	
Sum	1.00	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02	

Parameter changes are 1% for ω , 1% for α , and 5% for γ .

TABLE 5 Control coefficients of the enzymatic steps on the Fourier spectrum for model 2

Enzymatic step (<i>i</i>)	<i>G</i>					<i>T</i>			
	C_i^ω	$C_i^{A_0}$	$C_i^{A_1}$	$C_i^{A_2}$	$C_i^{A_2/A_1}$	$C_i^{A_0}$	$C_i^{A_1}$	$C_i^{A_2}$	$C_i^{A_2/A_1}$
V_{in}	0.78	-0.14	-0.24	-0.92	-0.68	0.91	0.51	-0.22	-0.73
k_1	1.11	-1.00	-1.87	-3.04	-1.17	-0.98	-1.65	-2.82	-1.17
k_p	-0.89	1.14	2.12	3.98	1.86	0.07	1.16	3.05	1.89
Sum	0.99	0.00	0.01	0.02	0.01	0.00	0.02	0.01	-0.01

Parameter changes are 5% for ω , G , and T .

models, the control by the enzymes on all different Fourier elements was distributed over the various processes. Negative control coefficients were calculated for each model, for various steps, and for all Fourier amplitudes. For the glucose influx step, the control coefficient for the frequency was positive for both core models, model 1 and model 2 (0.51 and 0.78, respectively) and negative for the more detailed model 3 (-1.54). Recently, we determined experimentally a control coefficient for the glucose transporter on the frequency of the limit cycle oscillations of +0.5 (Reijenga et al., 2001). A multitude of various molecular mechanisms can bring about the oscillations in glycolysis. A large difference in the frequency control exerted by the glucose transporter demonstrates that these mechanisms differ for the core models and a detailed model. It should also be noted that, for model 3, it was more difficult to choose a parameter change that was small enough for (local) linearity of the $\ln\text{--}\ln$ plot of X_j versus v_i and large enough for an accurate change in the variables. Both the average values and the amplitudes of the oscillations in the metabolites were small, and therefore numerical error was high.

Summation properties

Because of time scaling properties, the sums of the control coefficients with respect to frequency and amplitude have

been shown to equal 1 and 0, respectively (Giersch, 1988; Westerhoff and Van Dam, 1987; Westerhoff et al., 1990; Acerenza, 1990; Kholodenko et al., 1997). This is confirmed by Tables 4–6. The same tables suggest that the sum of the control coefficients with respect to the higher-order Fourier components should also equal zero, as should the sum of the control with respect to the waveform. The minor deviations from one and zero were in line with numerical error. We further tested the summation theorems by increasing all kinetic parameters simultaneously by 10%. As was expected, the frequency of the oscillations increased by 10%, whereas the average value, the amplitudes of the different Fourier components, and the waveform of the oscillations did not change. The sums of the changes after increasing all kinetic parameters simultaneously by 10%, are also listed in Table 6 (sum*).

Illustration of summation theorems

The summation theorems and the control coefficients are illustrated on model 2. For three different parameter sets, a Fourier spectrum of intracellular glucose was made. The first parameter set acted as the reference state ($V_{in} = 0.36$; $k_1 = 0.02$; $k_p = 6.0$; $K_M = 13.0$). For the second parameter set, all kinetic parameters were increased simultaneously by 10%, and, for the third parameter set, only the kinetic parameter for the second reaction (k_1) was increased by 10%. The first- and second-order Fourier components and the sum of the two components were plotted. The average concentration (A_0) was not taken into account. It is shown that, when all the rate constants were increased simultaneously by 10%, the amplitudes did not change (Fig. 3). This implies that the waveform (defined as A_2/A_1) did not change either. This illustrates the summation theorem for the waveform. In contrast, the frequency of both Fourier components increased by 10% (Fig. 3). When only the rate

TABLE 6 Control coefficients of the enzymatic steps on the Fourier spectrum for model 3

Enzymatic step (<i>i</i>)	C_i^ω	X_1 (ATP)				X_2 (FBP)			
		$C_i^{A_0}$	$C_i^{A_1}$	$C_i^{A_2}$	$C_i^{A_2/A_1}$	$C_i^{A_0}$	$C_i^{A_1}$	$C_i^{A_2}$	$C_i^{A_2/A_1}$
J_0	-1.54	0.83	2.94	3.87	0.93	0.99	4.18	3.78	-0.40
k_1	0.70	0.03	-1.24	-1.04	0.20	0.00	-1.45	-0.92	0.53
k_2	0.21	0.00	0.00	-0.02	-0.02	-1.00	-0.11	-0.07	0.04
k_3	-0.12	0.12	0.09	0.28	0.19	0.00	0.23	0.31	0.08
k_4	-0.11	0.00	0.37	0.24	-0.13	0.00	0.25	0.12	-0.13
k_5	0.01	0.01	0.01	0.02	0.01	0.00	0.02	0.01	-0.01
k_6	-0.30	0.24	0.36	0.70	0.34	0.00	0.60	0.67	0.07
k_7	1.65	-0.86	-2.00	-2.93	-0.93	0.00	-2.78	-2.72	0.06
k_8	0.19	-0.13	-0.20	-0.36	-0.16	0.00	-0.33	-0.36	-0.03
k_9	0.10	-0.08	-0.11	-0.22	-0.11	0.00	-0.19	-0.22	-0.03
κ	0.20	-0.16	-0.25	-0.48	-0.23	0.00	-0.41	-0.44	-0.03
Sum	0.99	0.00	-0.03	0.06	0.09	-0.01	0.01	0.16	0.15
Sum*	1.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.02

Parameter changes are 1% for A_0 and A_1 , and 5% for ω , A_2 , and A_2/A_1 .

*All kinetic parameters are increased simultaneously by 10% and the total change in A_0 , A_1 , A_2 , and A_2/A_1 is determined.

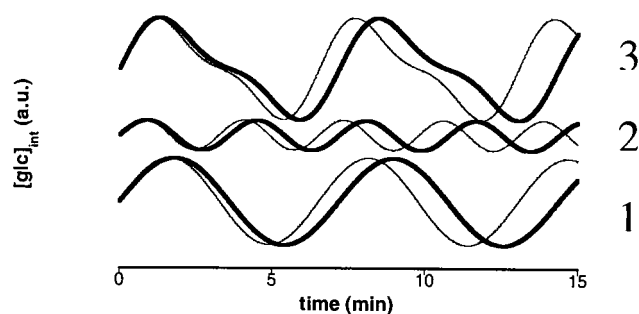


FIGURE 3 Output of the glycolytic model by Bier et al. (2000) A Fourier spectrum of the oscillating internal glucose concentration was taken and the individual components were plotted. Curves have been shifted relative to each other to separate the different components. First-order Fourier component (1), second-order Fourier component (2), and the sum of the two components (3). *Bold line*, control; *thin line*, all rate constants were increased by 10%. See the text for further explanation.

constant for the second reaction was increased by 10%, amplitude and frequency changed as calculated by the control coefficients for that step (Fig. 4). Here also the waveform of the signal was altered.

Control of the Fourier spectrum of the flux

In oscillating systems, not only the concentrations of metabolites vary in time, the fluxes through the enzymes are not constant either. As for the concentrations, these fluxes can be analyzed using the Fourier transform. In Table 7, we demonstrate such an analysis on the flux through PFK for model 1. The control on the average flux was completely determined by the (constant) input of substrate (cf. Teusink et al., 1996a) but the control on the amplitude of the oscillating flux and on the waveform of the oscillation was distributed. For the amplitude of the two Fourier compo-

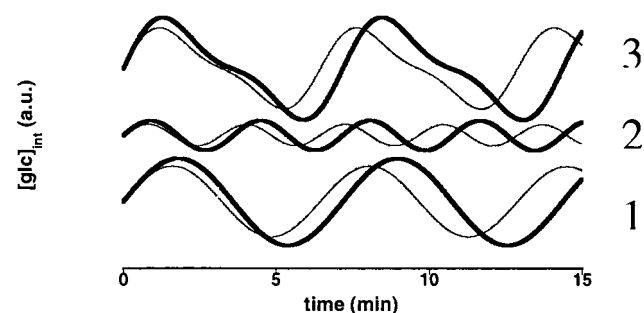


FIGURE 4 Output of the glycolytic model by Bier et al. (2000) A Fourier spectrum of the oscillating internal glucose concentration was taken and the individual components were plotted. Curves have been shifted relative to each other to separate the different components. First-order Fourier component (1), second-order Fourier component (2), and the sum of the two components (3). *Bold line*, control; *thin line*, the rate constant of the second reaction (k_1) was increased by 10%. See the text for further explanation.

TABLE 7 Control of the kinetic parameters of model 1 on the flux through PFK (second reaction)

Enzymatic step (<i>i</i>)	$C_{v_i}^{\omega}$	$C_{v_i}^{A_0}$	$C_{v_i}^{A_1}$	$C_{v_i}^{A_2}$	$C_{v_i}^{A_2/A_1}$
σ_1	0.52	1.00	0.76	0.16	-0.60
σ_M	0.40	0	0.23	0.88	0.65
k_s	0.08	0	0.01	-0.03	-0.04
Sum	1.00	1.00	1.00	1.01	0.00

nents, the total control added up to one, as it did for the control on the frequency and on the average value of the flux. However, the control on the waveform of the oscillation summed to zero.

Summation theorem for the phase of the oscillation

The Fourier spectrum also contains information on the phases of the individual Fourier components and on the phase differences among these components. The control coefficients for the individual enzymes could not be calculated because of the Fourier routine we used (i.e., the analysis was always started at the average value of the oscillation). However, for model 1, it was shown that, when all enzymes were increased by 10%, the phase difference between the second and first Fourier component did not change. This suggested that the sum of all the control coefficients on this phase difference should add up to zero.

Combined response theorem

For steady-state phenomena, response coefficients can be linked to control coefficients and elasticities. The combined response theorem quantifies the relation between the response of a variable toward a change in an external clamped substrate, the control of the linking enzyme (the enzyme for which the parameter is the substrate) on this variable, and the sensitivity of the enzyme toward the substrate. For the response of steady-state properties to external glucose, the combined response theorem reads

$$R_{\text{glucose}}^X = C_{\text{g.t.}}^X \cdot \varepsilon_{\text{glucose}}^{\text{g.t.}}$$

For dynamic phenomena, the metabolite concentrations are oscillating, and, therefore, this theorem can, at most, hold approximately. We set out to see to what extent the combined response theorem might still apply to oscillating phenomena. In model 1, the kinetics of the lumped reaction including glucose influx are defined as mass action ($k \cdot [S]$). Therefore, the elasticity (ε) for this step toward glucose is 1 and constant. This implies that, in this model, the response of the system toward a change in glucose (R_{glucose}^X) equals the control of the system by the glucose transport step ($C_{\text{g.t.}}^X$). Trivially the combined response theorem applies.

TABLE 8 Response of the Fourier spectrum to changes in external glucose concentration, control coefficients of glucose transport on the Fourier spectrum, and their ratio for model 3

	$R_{\text{ext,glc}}^X$	$C_{\text{g.t.}}^X$	$R_{\text{ext,glc}}^X/C_{\text{g.t.}}^X$
ω	-0.16	-1.20	0.14
A_0	0.52	2.79	0.19
A_1	0.66	3.41	0.19
A_2	0.68	3.18	0.21

To examine the more general case, we changed the kinetics of the glucose transport step for model 3 to reversible Michaelis–Menten kinetics ($V_{\text{max}} = 60$; $K_{\text{Ms}} = 2$; $K_{\text{Mp}} = 2$). We calculated the elasticity of the enzyme for external glucose using the equation,

$$\varepsilon_S^i = \left(\frac{\partial v_i}{\partial S_{\text{out}}} \cdot \frac{S_{\text{out}}}{v_i} \right)_{S_{\text{in}}(t)}.$$

Because the internal glucose concentration oscillated, we calculated v_i and ε_S^i during one period. The internal glucose concentration ranged from 0.18 to 1.75 mM, the glucose transport rate ranged from 46 to 55 mM min⁻¹, and the elasticity ranged from 0.11 to 0.25. Furthermore, we determined the response coefficients by changing the external glucose concentration and the control coefficients by changing the V_{max} of the glucose transport step (Table 8). The values for the ratio $R_{\text{ext,glc}}^X/C_{\text{g.t.}}^X$ fell within the range of values for the elasticity (0.11–0.25), but differed among the various Fourier characteristics. This shows that, although, for oscillating phenomena, the combined response theorem can be approximately true, it does not hold precisely, not even if written in terms of some sort of time average of the elasticity coefficient.

Response of the Fourier spectrum to changes in affinity of the enzymes and conserved moieties

For model 3 with reversible Michaelis–Menten kinetics for the glucose transport step, we determined the response of the Fourier spectrum to changes in affinity of the glucose transport step, as well as to changes in conserved moieties. The response and control coefficients, as well as the ratios between the response toward the K_{M} for external glucose and the control of glucose transport on the Fourier spectrum are listed in Table 9. The combined response theorem for the K_{M} value (Chen and Westerhoff, 1986) held approximately for oscillating phenomena,

$$R_{K_{\text{M}}}^X = -C_{\text{g.t.}}^X \cdot \varepsilon_{\text{ext,glc}}^{\text{g.t.}}$$

DISCUSSION

Dynamical systems are of great interest in both mathematics and the natural sciences. Jules Henri Poincaré founded the

TABLE 9 Response coefficients of Fourier spectrum to changes in K_{M} and conserved moieties, control coefficients of glucose transport on Fourier spectrum, and ratio of response toward K_{M} and control of glucose transport for model 3

	$R_{K_{\text{M}}}^X$	$R_{(\text{ATP}+\text{ADP})}^X$	$R_{(\text{NAD}+\text{NADH})}^X$	$C_{\text{g.t.}}^X$	$R_{K_{\text{M}}}^X/C_{\text{g.t.}}^X$
ω	0.16	-0.44	-0.11	-1.20	-0.14
A_0	-0.52	0.55	0.38	2.79	-0.19
A_1	-0.66	1.61	0.30	3.41	-0.19
A_2	-0.68	1.23	0.41	3.18	-0.21

modern *qualitative* theory of dynamical systems (Sarkaria, 1999) emphasizing issues such as whether, and for which parameter values, systems are stable, and what type of dynamics they exhibit. There is comparatively little attention for the precise dependence of the quantitative properties, such as material concentrations or fluxes of dynamic systems on molecular properties when the system remains within a single basin of attraction. For biological systems, precision and minor increases in functionality are important. Moreover, through molecular genetics and modern biochemistry, biological dynamic systems have become amenable to subtle manipulation of, in principle, each molecular (enzyme catalyzed) process (Jensen et al., 1993). In this way, biology is becoming a prime field for the further subtle analysis of dynamic systems (Westerhoff, 2001).

In view of the complexity of biological dynamic systems, a systematic way of analyzing them should then be useful. With some theoretical background behind us (Bier et al., 1996; Demin et al., 1999; Kholodenko et al., 1997, and references therein), we here operationalized such an analysis method. We did this through numerical experiments on a model of yeast glycolytic oscillations, because these are already relatively well defined, both theoretically and experimentally (Chance et al., 1964; Ghosh and Chance, 1964; Richard et al., 1994; Teusink et al., 1996a; Dano et al., 1999; Bier et al., 2000; Wolf et al., 2000). By describing a time-dependent periodic signal as a function of frequencies, using a Fourier transformation, again, a stationary description was obtained that was amenable to control analysis.

Often, periodic signals are described in terms of frequency, amplitude, and average value. Here, we also studied the waveform of the signal quantitatively by defining it as the ratio of the amplitudes of Fourier components. The phase difference between components gave additional information on the waveform of the signal. Therefore, questions such as, Is a periodic signal sinusoidal or not? and What controls the waveform of a signal? can now be answered in a quantitative way, both for measured and calculated signals.

As an example, the control of the different enzymes on the dynamics of the variables was determined for three different models of the same important process, i.e., glycolysis. Control was distributed among the enzymes. Distributive control of dynamics still has limited experimental

validation. Perhaps the most germane experimental study has been the determination of the control of frequency of glycolytic oscillations, the system also studied theoretically in this paper. The control of the glucose transport system on the frequency was shown to be +0.5. By implication, control of the frequency should be distributed among at least two molecular processes (Reijenga et al., 2001).

For frequency and amplitude, the distributive nature of control had already been shown by us for models of glycolysis (Teusink et al., 1996a). Here, this has now also been shown for waveform and phase difference. Moreover, the present paper constitutes a substantial improvement on the earlier approach in which we simulated the system and then tediously measured the effect on frequency and overall amplitude by direct inspection of the calculated data. Here we made this procedure systematic and objective by implementing a Fourier transformation, which then automatically led us to frequency and amplitudes. Only with this method could we reliably discuss the control of the waveform of the oscillations. The new approach is also more amenable to application to larger data sets. However, the empirical nature of our study does not provide us with an overall theory to aid in the understanding of oscillating phenomena. For recent developments in this, we refer to Kholodenko et al. (1997) and Demin et al. (1999).

The study of the control of the waveform was gratifying, because it showed, contrary to our expectations, that control on the form of the oscillations can be quite strong, even though the sum of the corresponding control coefficients is zero. This suggests that a living cell may not only regulate itself by adjusting the rate of its glycolytic flux or the frequency of oscillations therein (or in its cell cycle for that matter), but also by adjusting the form of the oscillations (or the time dependence of any dynamic phenomenon). Amplitude and form of oscillations can have strong implications for functionality and even for the thermodynamics thereof (Westerhoff et al., 1986; Berridge et al., 1998). More, in general, the possibility of identifying quantitatively which molecular players control dynamic games in the living cell may become crucial to progress in cell biology. The control and response coefficients introduced here to intracellular dynamics may serve as criteria. More and more the vast connectivity and multiplicity of the intracellular processes causes problems of analysis. When searching hard enough, any process seems to be influenced by almost any other process in the living cell. We have shown that some processes can be more important than others, i.e., not all control coefficients are the same in Tables 4–6. In contrast, the importance of molecular factors for a dynamic process may depend on the particular aspect of that process that is being considered. Enzymes that exert a strong control on frequency may not do so on average concentration or on the amplitude of the second Fourier component. Having shown this for models of yeast glycolysis, we anticipate that similar conclusions will emerge for the control of the cell cycle

(Tyson and Novak, 2001) and calcium oscillations (Höfer et al., 2001).

An important asset of the metabolic control analysis of steady-state systems has been that it exposed laws that should be obeyed by control coefficients. Of these laws the summation theorems have a counterpart in the control analysis of oscillatory systems. For the control of frequency, amplitude, and average value, the summation theorems had already been derived (e.g., Westerhoff et al., 1990; Bier et al., 1996; Giersch, 1988; Kholodenko et al., 1997). In the present paper, we came up with additional summation theorems on waveform and on phase difference, confirmed in three different mathematical models for yeast glycolytic oscillations.

These summation theorems are of considerable importance. First, where intuition may have suggested control to be constrained to a single pace-making step, the theorems show what the correct phrasing of the intuition should be. Control on frequency should indeed amount to a total of 1, but may well be distributed among all participating catalytic activities. Second, the theorems show that, with respect to the control of amplitude or waveform, the intuition is plainly misleading: control should not add up to 1, but to zero. Therefore, a system without any key step that controls the amplitude of an oscillation, may well exist. And, a system with a single such step cannot exist; if one step exerts a control of 1, then there must be other steps with control summing up to minus 1. Third, the theorems provide a prime rationalization for the observation that genes rarely have strong phenotypes. Also for dynamics, control is distributed and partial inactivation of a single process should, on average, have little effect (cf. Kacser and Burns, 1973).

Oscillations contain more information than steady-state phenomena. In view of the search for functions for silent genes (Raamsdonk et al., 2001), a focus on dynamic phenomena may become helpful, because analysis of steady-state variables does not always reveal phenotypes. The quantitative analysis of dynamic systems may provide information, answers, and additional biological problems. Discussing only the more regular of such systems, this operational analysis of steady oscillations is, perhaps, a first step toward a more general analysis method for such dynamical systems. It may well be possible to generalize the approach described here to other time dependent systems, such as those that arise upon perturbation of a steady system, e.g., when a growth factor is added to a mammalian cell (Acerenza et al., 1989; Heinrich and Reder, 1991; Kholodenko et al., 1999).

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